ANBCA2

ANALYTICAL BIOCHEMISTRY

An International Journal of
Methods in the Biological Sciences

EDITOR-IN-CHIEF

William B. Jakoby

EXECUTIVE EDITORS

Assistant to the Editor-in-Chief, William S. Allison

Shelby L. Berger
Ludwig Brand
Peter Brodelius
Wybren de Jong
Jack E. Dixon
Theodore Friedmann
Othmar Gabriel
William F. Harrington
Tony E. Hugli
Richard D. Klausner
John H. Law
Y. C. Lee
Walter M. Lovenberg
William D. McElroy
Sherie L. Morrison
Richard N. Perham
James F. Riordan
Enrique Rozengurt
J. Edwin Seegmiller
Clarence H. Suelter
Bert L. Vallee
Helen Van Vunakis
Arthur Weissbach

Alvin Nason, Founding Editor, 1960–1977
Nathan O. Kaplan and William D. McElroy,
Editorial Committee Co-Chairmen, 1978–1986

Volume 169, 1988

ACADEMIC PRESS, INC.
Harcourt Brace Jovanovich, Publishers
San Diego New York Boston
London Sydney Tokyo Toronto

Copyright © 1988 by Academic Press, Inc.

All Rights Reserved

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopy, recording, or any information storage and retrieval system, without permission in writing from the copyright owner.

The appearance of the code at the bottom of the first page of an article in this journal indicates the copyright owner's consent that copies of the article may be made for personal or internal use, or for the personal or internal use of specific clients. This consent is given on the condition, however, that the copier pay the stated per copy fee through the Copyright Clearance Center, Inc. (27 Congress Street, Salem, Massachusetts 01970), for copying beyond that permitted by Sections 107 or 108 of the U. S. Copyright Law. This consent does not extend to other kinds of copying, such as copying for general distribution, for advertising or promotional purposes, for creating new collective works, or for resale. Copy fees for pre-1988 articles are as shown on the article title pages; if no fee code appears on the title page, the copy fee is the same as for current articles.

0003-2697/88 $3.00

MADE IN THE UNITED STATES OF AMERICA

CONTENTS OF VOLUME 169

NUMBER 1, FEBRUARY 15, 1988

Review. Analytical Strategies for the Use of DNA Probes
Jayne A. Matthews and Larry J. Kricka 1

Fractionation of Type V Collagen from Carcinomatous and Dysplasic Breast in the Presence of Alkaline Potassium Chloride
C. Luparello, C. P. Rizzo, R. Schillaci, and I. Pucci-Minafra 26

Determination of Human Fibroblasts Metabolism *in Vitro* by Gas Chromatography–Mass Spectrometry of Cell-Excreted Metabolites
A. G. Antoshechkin, V. Yu. Tatur, O. M. Perevezentseva, and L. A. Maximova 33

Properties of the Clot Assay, an Easy Quantitative Assay of DNA Endonucleases
Margarita Cervera, Alberto Domingo, and Roberto Marco 41

Elsie 4: Quantitative Computer Analysis of Sets of Two-Dimensional Gel Electrophoretograms
Arthur David Olson and Mark J. Miller 49

Affinity Purification of Cytosolic Epoxide Hydrolase Using Derivatized Epoxy-Activated Sepharose Gels
Roger N. Wixtrom, Marilyn H. Silva, and Bruce D. Hammock 71

A Partition Assay for the Simultaneous Determination of Insect Juvenile Hormone Esterase and Epoxide Hydrolase Activity
Marla R. Share and R. Michael Roe 81

Identification and Measurement of Kynurenic Acid in the Rat Brain and Other Organs
V. Carlá, G. Lombardi, M. Beni, P. Russi, G. Moneti, and F. Moroni 89

Tritium Labeling of a Powerful Methylphosphonate Inhibitor of Cholinesterase: Synthesis and Biological Applications
A. Balan, I. Barness, G. Simon, D. Levy, and Y. Ashani 95

Affinity Chromatography of a Sequence-Specific DNA Binding Protein Using Teflon-Linked Oligonucleotides
Craig H. Duncan and Sharon L. Cavalier 104

An Assay for the α-Tocopherol Binding Protein Mediated Transfer of Vitamin E between Membranes
Carl P. Verdon and Jeffrey B. Blumberg 109

LC–EC Determination of Nucleotides and Nucleic Acids: Application to Enzyme Assays and the Analysis of DNA Fragments
David P. Malliaros, Mark J. DeBenedetto, Pamela M. Guy, Terrence P. Tougas, and Edwin G. E. Jahngen 121

Agarose Gel Electrophoresis of Denatured RNA with Silver Staining
Rose N. Skopp and Leslie C. Lane 132

Direct Nanogram Quantitation of Nucleic Acid and Protein with a Continuous-Flow Microcell
Jingdong Zhu, Abbas Rashidbaigi, and Sidney Pestka 138

Structure–Activity Relationships of Chloride-Sensitive Fluorescent Indicators for Biological Application
Reto Krapf, Nicholas P. Illsley, Hsien C. Tseng, and A. S. Verkman 142

Sequence Analysis of End-Labeled DNA Fragments by Solvolysis in Hot Aqueous Piperidine Solutions
Barbara J. B. Ambrose, Reynaldo C. Pless, and Mary E. Ayers 151

Incomplete Hydrolysis of the Calcium Indicator Precursor Fura-2 Pentaacetoxymethyl Ester (Fura-2 AM) by Cells
S. George Oakes, William J. Martin II, Carol A. Lisek, and Garth Powis 159

Two-Dimensional Protein Mapping by Gold Stain and Immunoblotting
A. H. V. Schapira and G. Keir 167
High-Performance Liquid Chromatography with Concanavalin A Immobilized by Metal Interactions on the Stationary Phase
Ziad El Rassi, Yunghuoy Truei, Yih-Fen Maa, and Csaba Horváth 172
Use of a Western Blotting Technique in the Purification of a Cysteine Proteinase Inhibitor *Marcus J. Korth, Donald J. Finn, and Gary L. Gustafson* 181
Dissociation of Proteinase–Inhibitor Complexes by Trichloroacetate
Gary L. Gustafson, Donald J. Finn, and Kamiar Moin 185
Multisample Parallel Array Fraction Collector: Application in DNA Alkaline Elution Studies *Meyrick J. Peak and William J. Eisler* 189
Simultaneous Electroelution and Concentration of DNA Fragments from Agarose Gels *William G. Stroop* 194
Quantitative Analysis of the Pyridinium Crosslinks of Collagen in Urine Using Ion-Paired Reversed-Phase High-Performance Liquid Chromatography
Darcey Black, Alexander Duncan, and Simon P. Robins 197
Novel Approach to Affinity Chromatography Using "Weak" Monoclonal Antibodies
Sten Ohlson, Arne Lundblad, and David Zopf 204
A Microtiter-Based Assay for Protein Kinase Activity Suitable for the Analysis of Large Numbers of Samples, and Its Application to the Study of *Drosophila* Learning Mutants *Joseph D. Buxbaum and Yadin Dudai* 209

NUMBER 2, MARCH 1988

Structural Elucidation of N-Terminal Post-Translational Modifications by Mass Spectrometry: Application to Chicken Enolase and the α- and β-Subunits of Bovine Mitochondrial F_1-ATPase
Bradford W. Gibson, Donald J. Daley, and Dudley H. Williams 217
Use of a Scanning Densitometer or an ELISA Plate Reader for Measurement of Nanogram Amounts of Protein in Crude Extracts from Biological Tissues
Sibdas Ghosh, Shimon Gepstein, John J. Heikkila, and Erwin B. Dumbroff 227
Selective Silver-Staining Methods for RNA and Proteins in the Same Polyacrylamide Gels *A. Paleologue, J. P. Reboud, and A. M. Reboud* 234
High-Performance Affinity Chromatography of Carbohydrate-Binding Proteins by Two-Stage Separation on a Resin Carrying a Number of Oligosaccharides
Susumu Honda, Kenji Suzuki, Shigeo Suzuki, and Kazuaki Kakehi 239
On the Use of Polyethylenimine as a Carrier for Protein Sequencing: Comparison with Polybrene *Jean Pierre Le Caer and Jean Rossier* 246
Comparison of Diazo-Coupling, Formazan, and Silver Staining Techniques for Visualizing Alkaline Phosphatase Isoenzymes after Electrophoresis in Homogeneous-Pore and Gradient-Pore Polyacrylamide Gels
Alan W. Hodson and Andrew W. Skillen 253
Detergent-Amplified Chemiluminescence of Lucigenin for Determination of Superoxide Anion Production by NADPH Oxidase and Xanthine Oxidase
J. Storch and E. Ferber 262
A Nonenzymatic Method for the Determination of Picomole Amounts of Lactate Using HPLC: Its Application to Single Muscle Fibers
W. S. Simonides, R. Zaremba, C. van Hardeveld, and W. J. van der Laarse 268

ynthesis of [γ-^{32}P]Thiamine Triphosphate
Ch. Grandfils, L. Bettendorff, C. de Rycker, and E. Schoffeniels 274
Jse of Ar^+ Plasma Etching to Localize Structural Proteins in Viruses: Studies with Adenovirus 2 *William W. Newcomb and Jay C. Brown* 279
)etermination of Interchain Crosslinkages in Insulin B-Chain Dimers by Fast Atom Bombardment Mass Spectrometry
Paul Toren, David Smith, Ronald Chance, and James Hoffman 287
Extraction and Measurement of Myocardial Nucleotides, Nucleosides, and Purine Bases by High-Performance Liquid Chromatography *Donald F. Hammer, Donald V. Unverferth, Robert E. Kelley, Paula A. Harvan, and Ruth A. Altschuld* 300
High-Yield and High-Degree Purification of Human α-Fetoprotein Produced by Adaptation of the Human Hepatoma Cell Line HEP G2 in a Serum-Free Medium
Mario F. Tecce and Benedetto Terrana 306
A Microassay for ATPase
Richard D. Henkel, John L. VandeBerg, and Richard A. Walsh 312
Graphical Analysis of Binding Data Reflecting Competition between Two Ligands for the Same Acceptor Sites *Simon J. Harris and Donald J. Winzor* 319
)irect Measurement of NAD(P)H:Quinone Reductase from Cells Cultured in Microtiter Wells: A Screening Assay for Anticarcinogenic Enzyme Inducers
Hans J. Prochaska and Annette B. Santamaria 328
Structural Characterization of Intact, Branched Oligosaccharides by High Performance Liquid Chromatography and Liquid Secondary Ion Mass Spectrometry
James W. Webb, Ke Jiang, Beth L. Gillece-Castro, Anthony L. Tarentino, Thomas H. Plummer, James C. Byrd, Susan J. Fisher, and A. L. Burlingame 337
Separation by High-Performance Liquid Chromatography of α- and β-Amino Acids: Application to Assays of Lysine 2,3-Aminomutase and Leucine 2,3-Aminomutase
D. John Aberhart 350
Enhancement of the Detection of Alkali-Resistant Phosphoproteins in Polyacrylamide Gels *C. Bourassa, A. Chapdelaine, K. D. Roberts, and S. Chevalier* 356
)etermination of Gallotannin with Rhodanine
Kenneth H. Inoue and Ann E. Hagerman 363
Seeing Gel Wells Well *Ivor Smith, Ruth Cromie, and Karen Stainsby* 370
Protein Analysis on Two-Dimensional Polyacrylamide Gels in the Femtogram Range: Use of a New Sulfur-Labeling Reagent *Ysander V. Boxberg* 372
Nonradioactive Labeling of Synthetic Oligonucleotide Probes with Terminal Deoxynucleotidyl Transferase
Abhay Kumar, Paul Tchen, Françoise Roullet, and Jean Cohen 376
Specific Killing of Human Endothelial Cells by Antibody-Conjugated Glucose Oxidase
V. R. Muzykantov, D. V. Sakharov, V. V. Sinitsyn, S. P. Domogatsky, N. V. Goncharov, and S. M. Danilov 383
Isolation of Nuclei from Melittin-Destabilized Cells
Peter J. Smith, Martin H. Friede, Brenda J. Scott, and Claus von Holt 390
Purification of Integral Plasma Membrane Proteins by Reverse-Phase High Performance Liquid Chromatography *Michael R. Sussman* 395
Measurement of Ionized Calcium in Normal Human Blood Platelets
Gundu H. R. Rao 400
Immunoblotting of Hydrophobic Integral Membrane Proteins
Gillian M. Small, Tsuneo Imanaka, and Paul B. Lazarow 405

Enhancement of the Sensitivity for *in Situ* Detection of Alkaline Phosphatase Using a Homopolymer of 2-Acrylamide 2-Methylpropanesulfonate
Hiroshi Yasue and Takashi Awata 410
Selective Quantification of Arachidonic Acid Hydroperoxides and Their Hydroxy Derivatives in Reverse-Phase High Performance Liquid Chromatography
Junji Terao, Selma Setsu Shibata, and Setsuro Matsushita 415
Scintillation Counting of ^{14}C-Labeled Soluble and Insoluble Compounds in Plant Tissue *David Sun, Larry E. Wimmers, and Robert Turgeon* 424
Purification Method of Bovine Rhodopsin Kinase Using Regeneration of Rhodopsin
Daisuke Okada and Atsushi Ikai 428

BOOK REVIEWS

The Yeasts, Vol. 1, *Biology of Yeasts,* Vol. 2, *Yeasts and the Environment.* Edited by A. H. Rose and J. S. Harrison Reviewed by *Enrico Cabib* 432
Optimization of Chromatographic Selectivity. By P. Schoenmakers
Reviewed by *John K. Baker* 432

AUTHOR INDEX FOR VOLUME 169 433

The Subject Index for Volume 169 will appear in the December 1988 issue as part of a cumulative index for the year 1988.

INFORMATION FOR AUTHORS

Analytical Biochemistry is an international journal that publishes original articles on methods and methodology of interest to the biological sciences and all fields that impinge on biochemical investigation. In addition to the expected techniques that apply to biochemical preparations and analysis, the following should be considered as within the scope of the journal:

— Cell biology and cell, tissue, and organ culture methods that are of general application.

— Membranes and membrane proteins. These methods form a major section of interest.

— Procedures of interest in the field of molecular genetics including those necessary for cloning, sequencing, and synthesis of nucleic acids and for mutagenesis.

— Purification of enzymes and other proteins, but only if the methods are both novel and applicable generally, i.e., the interest is in a means of purification rather than in the protein that is being purified.

— Immunological techniques both analytical and preparative, if they are of general applicability by biochemists. Immunoassays are included but only if a unique approach is introduced rather than the determination of a substance not previously assayable.

— Pharmacological and toxicological research techniques that offer a biochemical approach.

— Review articles dealing with timely topics. Although these are generally invited, unsolicited outlines or manuscripts will be considered.

Manuscripts are accepted for review with the understanding that the same work has not been and will not be nor is presently submitted elsewhere, and that its submission for publication has been approved by all of the authors and by the institution where the work was carried out; further, that any person cited as a source of personal communications has approved such citation. Written authorization may be required at the Editor's discretion. Articles and any other material published in *Analytical Biochemistry* represent the opinions of the author(s) and should not be construed to reflect the opinions of the Editor(s) and the Publisher.

Authors submitting a manuscript do so on the understanding that if it is accepted for publication, copyright in the article, including the right to reproduce the article in all forms and media, shall be assigned exclusively to the Publisher. The Publisher will not refuse any reasonable request by the author for permission to reproduce any of his or her contributions to the journal.

Address. Manuscripts should be submitted in quadruplicate to *Analytical Biochemistry*, Editorial Office, Seventh Floor, 1250 Sixth Avenue, San Diego, California 92101. In the case of rejected manuscripts, only one copy of the manuscript will be returned to the author.

An extended Information for Authors is published in each July issue. Reprints are available from the Editorial Office. Authors should consult this Information for details on nomenclature, IUPAC-IUB rules and recommendations, and accepted abbreviations.

Manuscripts should be concise and consistent in style, spelling, and use of abbreviations. In order to facilitate the processing of manuscripts, the journal requests that at least four copies be submitted, each copy to include all figures (originals or glossy prints) and tables. The original copy must be typewritten, double-spaced, on one side of white bond paper, about $8\frac{1}{2} \times 11$ inches in size, with one-inch margins on all sides. Each manuscript should have a separate title page noting title of article, authors' names (without degrees), a running title (not exceeding 55 letters and spaces), an abstract (250 words), and the address to which proof should be mailed. In creating a title for your paper, please omit words such as *novel*, *rapid*, *improved*, *simple*, *sensitive*, *efficient*, *convenient*, *new*. Each is felt to be redundant and actually slows down the reader who is scanning the article titles and wants to know more about the method itself. Please substitute informative words that will give readers a more complete idea of the usefulness of your paper with regard to the limitations of their equipment or sample type. For example, what *kind* of method are you describing? Abbreviations should not be used in the article title or running title.

Please number all pages of the manuscript.

Authors should indicate the following on the title page: (1) the appropriate subject category for listing in the Table of Contents; (2) six descriptive key words for computer-aided selection of reviewers. (Please see the Appendix at the end of the extended Instructions to Authors published in the July 1, 1986 issue for a list of recommended key words.) These words will also be used in the preparation of the annual cumulative subject index for the journal.

Text. The suggested organization of an article is: abstract; introductory statement; Materials and Methods; Results; Discussion; Acknowledgments; References. Some of these sections may be combined if the presentation is thereby made clearer or more effective. Although this is the general form, the journal will accept short papers of approximately two printed pages that are arranged without specific sections except for a short abstract.

Names of chemical or organic substances should follow the recommendations of the IUPAC–IUB Combined Commission on Biochemical Nomenclature (CBN) as published in the extended Instructions for Authors, appearing in the July issue of this journal.

Authors should draw attention to any particular chemical or biological hazards that may be involved in carrying out the experiments described. Any relevant safety precautions should be

described; if an accepted code of practice has been followed, a reference to the relevant standards should be given.

Figures should be numbered consecutively with Arabic numerals in order of mention in the text: each figure should have a descriptive legend. Legends should be typed together on a separate sheet, double-spaced. All illustrations should be in finished form ready for photoreproduction. A convenient size for drawings is $8\frac{1}{2} \times 11$ in. (21×27.5 cm). Drawings should be made with India ink on tracing linen, smooth surface white paper, or Bristol board. Graph paper if used should be ruled in blue. Grid lines that are to show in the final reproduction should be inked in black. Glossy prints properly prepared are acceptable.

Photographs must be kept to a minimum. All photographs should be submitted as single glossy prints with strong contrasts and not mounted on cardboard; the magnification should be indicated by a scale where possible. **Simple histograms should be avoided. A table or a paragraph in the text is preferred.**

Illustrations in color can be accepted only if the authors defray the cost.

Tables should be typed on separate pages, numbered consecutively with Arabic numerals in order of mention in the text. Each table should have a short explanatory title.

Authors may wish to submit complex tables as camera-ready copy. In this way, they are spared tedious proofreading and the chance of error is reduced. Tables submitted as camera-ready copy should be typed single-spaced whenever possible, and preferably submitted as 5×7-in. glossy prints.

Footnotes should be designated in text by superscript numbers and typed on a separate sheet. Footnotes to tables should be identified with lowercase italic superscript letters and placed at the bottom of the table.

References to the literature should be cited in the text by Arabic numerals in parentheses and listed at the end of the paper in consecutive order. Abbreviations of journal titles should follow the style used in *Chemical Abstracts Service Source Index,* 1985. Please note style of capitalization and punctuation for journal articles and edited books in the following examples:

1. Jackson, G. E. D., and Young, N. M. (1987) *Anal. Biochem.* **162,** 251–256.
2. Bovey, F. A., and Jelinski, L. W. (1983) Chain Structure and Conformation of Macromolecules, p. 150, Academic Press, New York.
3. Britton, G. (1985) *in* Methods in Enzymology (Law, J. H., and Rilling, H. C., Eds.), Vol. 111, pp. 113–149, Academic Press, Orlando, FL.

Proofs. Proofs will be sent to the authors with reprint order forms. Fifty reprints of each article are provided free of charge. Additional reprints may be ordered using the form provided.